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DECLARATION UNDER 37 C.F.R. §1.132

I, Terry Potter, hereby declare as follows:

1. I am a co-inventor of the data correlation and analysis tool described and claimed in the above-identified patent application serial number 10/821,750 (“the ‘750 application”). I earned my PhD in System Science with a major in Computer Science from SUNY Binghamton in 1987.
2. I have reviewed U.S. Patent No. 5,517,251 (Rector) and conducted a search for information regarding IFFPHYS described in Rector at column 6, lines 37-46. Enclosed herewith as Exhibit A is an article I found: Rector, David M. et al., “Continuous Image and Electrophysiological Recording with Real-Time Processing and Control,” METHODS 25, 151-163 (2001).
3. An associative mapping as described in the ‘750 application has significant advantages when stored as a table. The associative mapping can be accessed by the content of any selected rows or columns. Additional rows or columns can

be added at any time to provide additional response variables or to include an additional stimulus stream. For example, “newly created variables 106 can be one variable, such as an average, or a multitude of variables, such as a set of values of how far each process variable is from the mean.” (‘750 application, page 9, lines 25-27) Also the time slices of a video signal taken at a different angle from the original video signal could be added onto the table and thus be related to the response variables. Searching through any given row or column for variables meeting a search criteria can be accomplished very quickly.

4. The IFFPHYS file format of Rector, on the other hand, is simply a flat file for continuous image and electrophysiological data recording. The data is mixed or interleaved with the video signal producing one combined signal in the IFFPHYS file format.
5. Searching through an IFFPHYS file would require reading through the entire signal video and data, since they are mixed or interleaved into a single file.
6. Once the file has been created, Rector shows no accommodation for mixing in additional data. Indeed, space for data is limited to that which is available in the video signal, whereas the associative mapping table format of the ‘750 application allows unlimited additions of data. Should the computer memory be exhausted, more memory can be added and the table format permits the addition of more data.
7. The IFFPHYS file format mixes a plurality of data signals into one video file. The data is inserted into the vertical blanking interval and other available spaces in the video signal. Given the format of the IFFPHYS file a second video signal

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does not make sense and Rector does not suggest any more than the one video signal.

8. I hereby declare that all statements made of my own knowledge herein are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application in connection with which this declaration is being submitted to the Patent and Trademark Office, or any patent issued thereon.

3-19-2010
Date

Terry Potter
Terry Potter

Continuous Image and Electrophysiological Recording with Real-Time Processing and Control

David M. Rector and John S. George¹

Physics Division, Biophysics Group, Los Alamos National Laboratory, Los Alamos, New Mexico 87545

Collecting continuous video together with multichannel electrophysiological data and other experimental modalities requires high bandwidth and storage capacities, as well as accurate synchronization to detect correlations between different recorded events. Often, experiments are highly complex, with many variables requiring immediate analysis and feedback during the course of the experiment. In addition, output channels require real-time control with high time resolution. We have explored several approaches to a system that can perform the above functions. The design of our system considered a number of issues, including time intervals between control and acquisition events, longest continuous recording period, data transfer bottleneck considerations, file archiving and format, and real-time display and processing. To demonstrate the system, we describe an experiment for characterizing rapid evoked scattered light changes in neural tissue, *in vivo*, using simultaneous electronic image acquisition and electrophysiological recording. © 2001 Elsevier Science

The ability to record from large neural populations simultaneously is necessary to assess how neurons behave in a network. Although multiple electrode techniques have grown rapidly from the use of a few electrodes to a hundred or more electrodes in a tight array (1), electrical measurements using such arrays continue to be invasive and of limited spatial resolution. In principle, a tightly integrated multiplexed amplifier system might provide a practical cost-effective data system; however, existing systems allow acquisition of at most a few dozen parallel channels.

From very early in the history of neuroscience investigation, optical microscopy has been used to study the

structure and function of neural tissue. Optical methods have a number of advantages; they can sample the relevant range of spatial and temporal scales, and provide information on microanatomy as well as biochemical and physiological processes. A number of investigators have observed reflected light changes from activated cortical surfaces and exploited the phenomenon to generate spatial organization maps of columnar structures in visual cortex that complement electrophysiological recording (2–4). Others have visualized sensory activation patterns in human temporal cortex (5) and rodent sensory cortex (6, 7).

Although optical measurements can resolve single action potentials in the giant squid axon and crab nerve (8, 9), these demanding measurements have previously only been accomplished with sensitive single-channel technologies. Most investigators interested in network dynamics have used dyes sensitive to membrane potential or intracellular ion concentrations, and employed photodiode array detectors (8, 10). Recent studies use fast CCD cameras with less toxic dyes (11); however, associated instrumentation remains expensive with limited functionality. Delivery of dyes is difficult and photoreaction may cause damage.

Although imaging is a useful technique, a network in its basic form contains patterns of communication between neurons in a network. The network has input and output projections that collect information and influence structures outside the network (Fig. 1). To fully understand the behavior of a given network, we must control input to the network or perturb neurons within the network directly and simultaneously record effector responses of the imaged network using multichannel analog techniques. There are many basic requirements that a data acquisition and control system must meet to perform these experiments. A fast, sensitive video camera is needed to image the network activity. Digital

¹ To whom correspondence should be addressed at Biophysics Group, P-21, MS-D454, Los Alamos National Laboratory, P.O. Box 1663, Los Alamos, NM 87545. Fax: (505) 665-4507. E-mail: jsg@lanl.gov.

and analog outputs are used to perturb the network in a systematic way and a multichannel analog input system is required to record the effector responses. These components need to be synchronized, with a well-defined time granularity, archived onto a mass storage device, and displayed and processed in real time so that digital and analog controls can respond to the patterns observed within and external to the network, effectively forming a closed loop.

Recording video and many electrophysiological channels simultaneously poses a significant challenge to data acquisition systems. We were unable to identify a commercial product to perform fast video digitizing that would also acquire analog channels for electrophysiology. Some systems specialize in digitizing a few channels very quickly (1 to 4 channels at 100 MSPS, 8 bits), or in digitizing a large array of separate input channels (256 channels) but at relatively slow rates (e.g., an aggregate rate of 1 MSPS). Also, many multichannel systems use multiplexing switches with very slow on/off times (greater than 1 μ s), thus reducing the system's overall ability to acquire multiplexed channels at high rates.

Several products claimed to deliver the performance

we required; however, the actual limitations were disappointing, and the systems could not perform the desired tasks. Rapid and very long data streams were difficult to handle. Data could not be simultaneously acquired and displayed at maximum acquisition rates. Limited on-board buffer memory made long continuous data streams impossible. Additionally, video acquisition and high-resolution experimental control required separate systems or boards which were difficult or impossible to synchronize.

For these reasons, we developed methods for multiplexing electrical and video information. The easiest method involved building a simple switching circuit so that a portion of each video line was replaced with concurrent electrophysiological values, resulting in a signal that could be captured with a video digitizer and parsed for video and electrophysiological data (13, 14) (U.S. Patent 5,517,251). This procedure worked well for synchronizing slow electrical signals (<200 Hz) with image data at real-time video frame digitizing rates (60 fps); however, this method had limitations for higher-performance frame capture (>100 Hz) and faster electrical signals (>1000 Hz).

A variety of commercial video digitizers provide the least expensive and easiest method for digitizing video signals multiplexed with analog channels. Unfortunately, there are also several limitations with inexpensive, consumer-grade digitizing boards. Many manufacturers claim 8-bit digitizing, but store only 5 or 6 bits of gray-scale information in memory. Low dynamic range simplifies video compression video for home movies, but precludes detection of small signal changes. We have found that any type of lossy compression scheme such as "mpeg" creates artifacts in low-contrast images when they are subtracted or ratioed.

Both consumer and scientific grade boards have a limited throughput for continuous, full, and uncompressed video frames (e.g., 3 fps). Some scientific grade boards with PCI interfaces can stream video into motherboard memory, but usually only for a limited amount of time and at the expense of all other processor operations. Further, the NTSC standard limits the total frame rate to 60 fps and a maximum dynamic range of 8 bits. To reduce the total data rate and to free resources for other tasks, a few boards allow for selection of a subregion of interest. Despite the inability to multiplex electrophysiology with a standard video signal, commercially available systems remain inadequate for high-performance recordings.

We have recently demonstrated dynamic imaging of intrinsic optical signals *in vivo* (12). In addition to slow changes associated with hemodynamic activation, we have observed fast light scattering changes associated

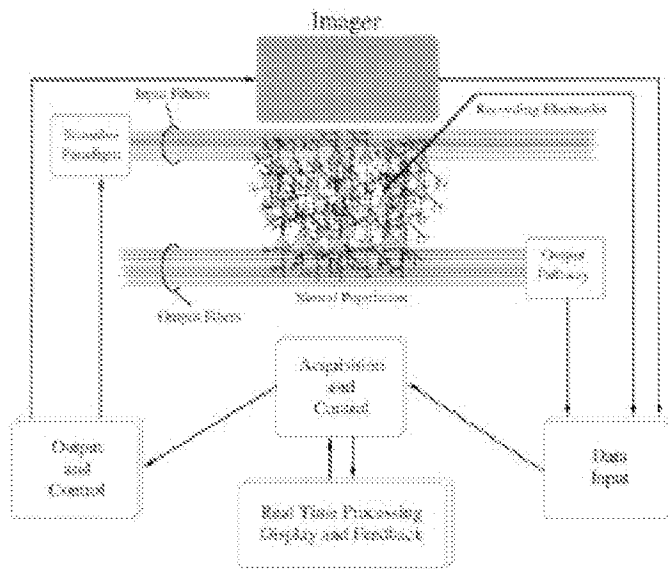


FIG. 1. A typical imaging paradigm includes a sample neural population with input fibers and output fibers (or effectors). An optimal system for recording the behavior of the neural population includes electrical recording electrodes, an imaging device, and detection of output pathways from the population. Dynamic feedback and perturbation of the system can be achieved through a stimulus paradigm. The system for performing these tasks needs to accept input data and simultaneously to control output parameters such as CCD camera timing signals and stimuli. An effective system would have integrated acquisition and control as well as real-time processing for on-line display and feedback.

with the physical processes of neurophysiological activation. The basic requirements for a system to record these signals (outlined in Table 1), include 100 to 1000-fps video resolution, 12 to 16 bits of dynamic range, 16 to 256 electrophysiological channels at 1 kHz each channel, and 25-ns timing granularity to control the CCD camera in real time. The continuous data stream produced is typically 1 to 64 MB per second, limited mainly by the rate at which we can archive the data. On-line display typically shows images and histograms for one of every three video frames. On-line time-triggered averaging captures every sequence generated. We use inexpensive but high-performance CCD chips for our miniature imagers and have developed a cost-effective, high-performance system for video, electrophysiology, and other data acquisition and for instrument and experiment control.

The digitizing system we developed performs all of the operations described above. Data inputs include 1 to 32,000 electrophysiological channels and one or more video signal inputs at 100 to 10,000 fps. The aggregate data throughput rate is currently limited to 32 MS/s (64 MB/s); however, migration from the PCI interface to the faster Compact-PCI or PCI-X bus would double or quadruple that value. The digitizer, analog multiplexer, and video control outputs are controlled synchronously by a field programmable gate array (FPGA). This device is configured as a state machine that executes a sequence of microcode instructions optimized for data acquisition and control. Data are double buffered so that when one buffer is full, the data banks are switched and the host downloads the data. The host archives the data to a large hard disk, performs some calculations on the data (e.g., image sequence averaging and histogram generation), and displays the data. The software is written so that separate tasks are divided into separate

functional processes, with interprocess communication through network socket protocols. This allows different tasks to be performed by one CPU, multiple CPUs, or many computers connected through a network.

DATA ACQUISITION SYSTEM

We developed an inexpensive solution to meet our data acquisition and control needs (Fig. 2). A host computer with PCI interface capabilities, fast display, and mass storage devices is configured with a high-performance operating system such as Linux. Other operating systems including Windows, MacOS, and OS2 are too slow and cumbersome to handle high-volume continuous data streams along with on-line display. Software is written in "C" using Unix socket protocols to make data available to internal processor tasks, or through a fast ethernet connection to other computers in a multitasking environment. Built-in DMA can transfer data from the acquisition board to the host computer through the PCI bus at 132 MB/s or through a Compact PCI bus at 528 MB/s or higher. DMA transfers occur in parallel with CPU operations, freeing the CPU for simultaneous data processing.

TABLE 1

Basic and Ideal Requirements for an Acquisition and Control System^a

Parameter	Basic specification	Typical performance
Electrophysiological channels	16	256 (up to 32,000)
Electrophysiological channel rate	100–1000 Hz	100–32,000 Hz
Video pixels per frame	10,000	10,000–250,000
Bit resolution	12 bits	16 bits
Video rate	100 fps	100–5,000 fps
Aggregate data throughput	1.0 MB/s	64 MB/s

^a Data throughput is calculated from electrophysiological and video parameters. The typical performance is achieved with a multiprocessor computer using RAID archive hardware.

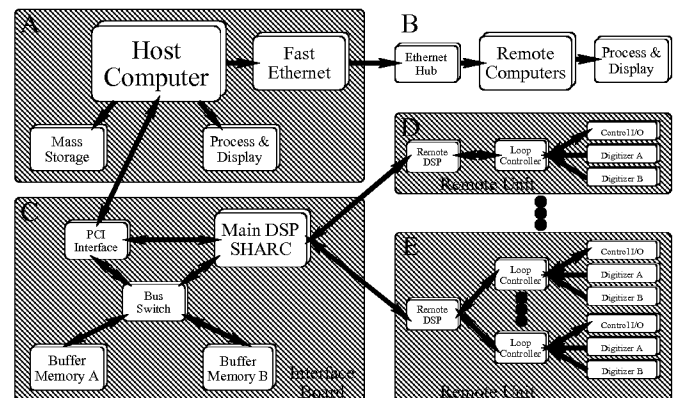


FIG. 2. Block diagram of the acquisition and control system. A host computer running the LINUX operating system communicates with separate processes through network sockets for archiving onto a mass storage device, on-line processing and display, and fast ethernet connection to remote computers. An interface board containing a PCI interface, double-buffered memory banks, and an Analog Devices SHARC DSP chip plugs into the PCI slot of the host computer. As many as six remote boards can be connected to the main interface board, each of which uses another SHARC DSP and a loop controller chip for output control and acquisition. Each remote unit can have up to four loop control chips, each of which can directly accept two 16-bit data paths. The system is designed for continuous high-throughput data streams with many parallel tasks and data paths.

MAIN INTERFACE BOARD

The main interface board outlined in Fig. 2 consists of five main components. The current prototype board fits on a full-length PCI card. Optimization of the PCI interface chip and discrete gates will reduce the size of the main card to a half-size card, or the components of the remote board can be added to a full-length single board.

A PCI interface chip controls DMA transfers to the host and maps memory locations for I/O ports, data buffer memory, and DSP memory. A 54-bit bus switch is responsible for swapping the data and address busses of the PCI interface chip and DSP with two banks of data buffer memory. The bus switch introduces only 0.3 ns of signal delay to the signal path and can switch the data and address busses within 7 ns. The bus switch allows data collection to one bank while the host is reading data from the other; when the data collection buffer is full, the banks are swapped, and continuous data transfers are possible. Buffer memory is 32 bits wide and can be upgraded from 64K words to 16M words. Typically, 1 to 5 s worth of data is stored in a buffer before switching occurs. The host can also request a buffer switch whenever it is ready for more data; thus, higher-performance computer systems have the capability to display more of the data with less buffer pipeline delay.

Several standard computer interfaces are available to transfer data from the acquisition system to the host computer. Newer boards use the PCI interface with a 32-bit bus and 33-MHz clock for a maximum transfer rate of 132 MB/s. More recent versions of the PCI interface are similar to the original PCI standard, but with enhancements. The new PCI-X and Compact-PCI interfaces have a 64-bit bus, and can have a clock rate of 66 or 133 MHz for a maximum transfer rate of 1 GB/s. PCI-X is currently being developed for Desktop PCs, while the Compact-PCI interface is currently implemented in rack-mount systems.

For high data acquisition rates, cabling between the acquisition system and the host computer becomes a major issue. Faster data rates over copper wire cable require wider data busses and/or higher transfer clock rates. Wider cables result in a bulky system while higher data rates often result in impedance issues that can degrade the reliability of the data transfer. For these reasons, we believe optical fiber will be the transfer medium of choice in the near future. Already, many

computer subsystems have the capability of transferring data through optical fiber at high rates. For example, fiber-link hard drive controllers and high-speed fiber ethernet controllers are capable of transferring greater than 10 GB/s.

OPTIONAL DSP CHIPS

The acquisition hardware can connect directly to the bus switch to transfer collected data; however, utilization of the Analog Devices SHARC digital signal processing chip provides many advantages. First, parallel tasking within the DSP chip allows preprocessing of incoming data such as digital filtering and image analysis in parallel with data transfer. The SHARC DSP provides two data move operations, two math operations, and one compare operation in every clock cycle, making it the highest-performance DSP available. Math capabilities include integer and floating point operations. Optimized instructions for Fourier analysis allow real-time digital filtering of electrophysiological channels. The SHARC DSP chips also provide six high-speed Link ports for transferring data to and from remote acquisition boards up to 10 ft away from the host system. Such an arrangement allows flexible modular configuration of acquisition components and simplifies the design of complex systems. SHARC chips are among the least expensive DSPs available, ranging in price from \$30 to \$300 depending on clock speed, internal SRAM, and Link port capabilities.

REMOTE BOARD

Up to six remote boards can be connected to the main board through each of six Link ports available on the DSP chips. The remote board consists of analog-to-digital subsystems and a DSP chip for data transfers to the main board and optional data preprocessing. Up to four FPGA-based loop control chips can be connected to the DSP chip. Each loop control chip has 64 bits of digital output for process control, 16 bits of timed interval triggers, and two dedicated 16-bit input registers. Each loop control chip can independently control and acquire data from a variety of sources; for example, a single remote board may have two loop control chips, one for controlling and acquiring from a CCD camera and the other for capturing electrophysiological data. Remote boards can be up to 10 ft away from the host

system and allow flexibility in modular acquisition stations that report back to the main host computer.

LOOP CONTROL CHIP

The internal structure of the loop control chip is outlined in Fig. 3. An FPGA contains a simplified microprocessor core that executes instructions stored in an external 64K by 32-bit SRAM bank. In the current system, we use the QL4036-2 (Quicklogic, CA) device which

contains 36K gates and 12K built-in SRAM. We chose Quicklogic devices over other manufacturers such as Xilinx and Altera for two main reasons. Early prototypes used Xilinx and Altera devices; unfortunately, these FPGAs were too slow for adequate real-time, non-pipelined control logic (10 MHz). Also, logic blocks within Xilinx and Altera devices were not optimized for high-speed counters and high-level multiplexing. Quicklogic devices provided the fastest logic implementation of any of the devices we evaluated (80–100 MHz) and required the fewest logic blocks to implement the

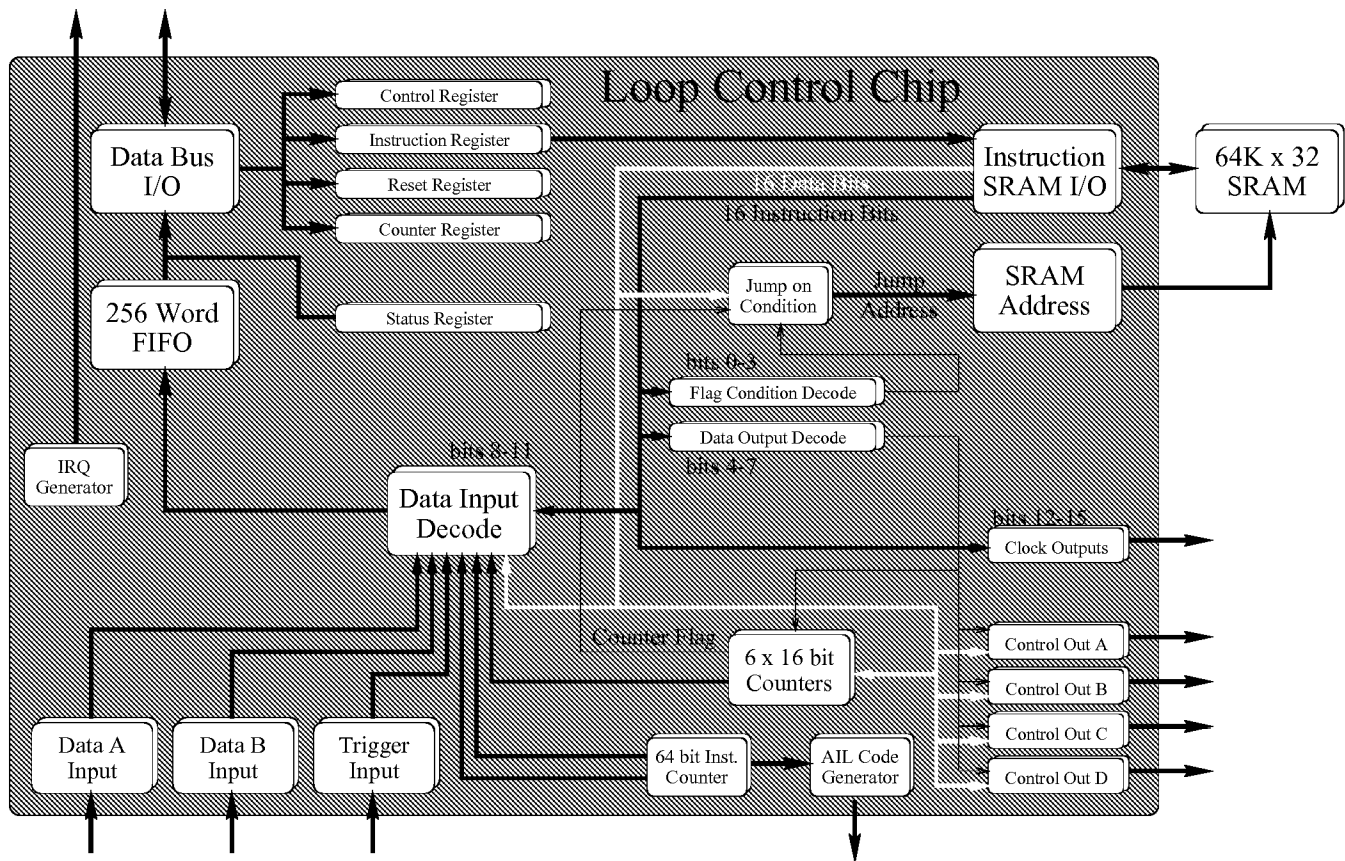


FIG. 3. The internal structure of the loop control chip contains several main components. Communication to the SHARC DSP is achieved through a 32-bit data I/O bus and interrupt generator (IRQ). Four 32-bit registers control components within the chip. The control register manages various processes of the chip, including programming of the instruction SRAM and setting up of the IRQ mask. The instruction register connects directly to the instruction SRAM I/O bus and is used to transfer instructions. The reset register is responsible for clearing flags within the chip, such as "FIFO full" and IRQ codes. The counter register is used for process counters. A 32-bit FIFO (first-in, first-out) memory bank is used to buffer incoming data, and a 32-bit status register can inform the SHARC DSP of the status of internal variables such as flag states and error conditions. The instruction SRAM I/O and SRAM address components of the chip are responsible for programming the microcode instructions into 64K × 32-bit-wide external memory. During operation of the chip, the SRAM address is incremented after each cycle, unless a jump location is requested. The 32-bit instruction word is divided into two parallel 16-bit paths. The 16-bit data path is used to output control signals to one of four output control registers, determine the "jump to" address, output directly to the acquisition data stream, or program one of six 16-bit counters. The 16-bit instruction path simultaneously performs four tasks. Bits 0–3 define a flag condition code that determines whether a jump will occur, bits 4–7 decide where the 16-bit data word will go, bits 8–11 determine which input path will be used in the acquisition data stream, and bits 12–15 are four dedicated output lines for high-speed signal control. A 64-bit instruction counter increments once with each instruction cycle, keeps track of absolute running time, and generates a plottable "AIL" time code for polygraph records. Six 16-bit counters are used to keep track of the number of times an instruction is executed and also to decide whether a jump occurs.

microprocessor core. The speed advantage is mainly at the sacrifice of reprogrammability; Quicklogic devices can be programmed only once. However, we never used the reprogrammability of the other devices since the initial design was flexible enough to meet our application needs.

A data bus I/O block handles all transactions between the internal components of the loop chip and the SHARC DSP. The SRAM instruction bank is programmed via the instruction register, and instructions begin executing on completion of the program download. After each clock cycle, the SRAM address is incremented, and a new instruction is executed. The 32-bit instruction word is divided into two 16-bit paths similar to the Harvard Architecture, where data and instructions appear in parallel. The 16 data bits can define one of four operations: (1) jump to address if conditions are met, (2) control data output, (3) load counter values, or (4) place 16-bit values within the collected data stream. The 16 instruction bits are divided into four parallel operations: (1) flag condition decode, (2) data output decode, (3) data input decode, and (4) four dedicated output control lines for high-speed timing signals such as digitizer clocks.

The loop control chip runs at 40 MHz and allows parallel output, input, and control in a user-defined sequence with well-defined timing not possible with other microprocessor cores. This arrangement allows us to write control sequences that are downloaded into the SRAM chip to control a CCD camera and a variety of other hardware and simultaneously digitize CCD output and a large number of electrophysiological channels. Digitized data are directed into one of the double-buffered SRAM banks. When a bank is full, it is immediately swapped, the host is notified of a full buffer, and digitizing continues into the new buffer.

CCD CAMERA

The current CCD camera chip (TC211, Texas Instruments) has a maximum pixel readout rate of 10 Msps, with a maximum full-frame rate of 250 Hz. Skipping every other readout line increases the frame rate to 500 Hz. Currently, the TC211 chips have a 1.5-LSB noise level at a maximum readout rate of 10 Msps and 12 bits digitization. An improved camera (Felix) designed for us by Dalsa Inc. of Canada has the ability to read out the pixels at 30 Msps and has a high signal-to-noise ratio. The faster readout rate will increase the maximum frame rate to 1500 Hz and improve the temporal resolution of the data.

DATA ARCHIVING

We prefer to archive as much of the raw data as possible; however, we encountered a significant problem in identifying storage space for long continuous streams of high-density data. In some circumstances, the data can be compressed to a certain degree, saving archival space, as well as reducing the load at potential bottlenecks. We have experimented with a number of compression algorithms, including byte packing, jpeg, and various lookup table algorithms. In general, the host spent more time compressing the data than was gained by the effort. Typically, a 10% reduction in data was achieved at best. Image data, if black level and gain are properly optimized, are not very compressible except by lossy techniques. In our experience, any type of lossy compression introduces artifacts that appear on images processed by subtraction or ratio routines.

FILE FORMAT

The file format should be easy to use and contain complete embedded documentation, so that someone coming back to the data 100 years later could interpret the data stream. There trade-offs to be considered in the file format. The easiest data to access are in some kind of raw form, for example, a raw binary sequence of digitized values. However, the program interpreting the data would be lost if you changed the number of input channels or the acquisition rate midstream. Someone perusing the data 100 years (or 100 days) later would be at a loss to interpret the data. On the other extreme, a data set with too much descriptive information is wasteful, bulky, difficult, and slow to parse.

A good compromise is to have a dataset with occasional headers describing the data to follow, with enough information to let the parser know about the format of the data. Such a file format would also have to be flexible enough to handle a great variety of data types, specifications, and styles. We chose to use the IFFPHYS (Interleaved File Format for Physiological Data, D. Sirag) format due to its consideration of all these features.

IFFPHYS is a standard interchange format for measured data from a wide variety of data acquisition systems. The data set is frequently too large to fit into available random access memory, and thus usually must be processed in blocks from a mass storage device with data from all channels available in each block. Files containing the physical data should be able to preserve all related information in a standard manner so that the data are available to a variety of analytic

programs. The objective of IFFPHYS is to specify a uniform manner to record physical data into a file and yet accommodate the wide variety of physical data and instruments with which measurements are made. This goal is accomplished by allowing the data to be stored in blocks preceded by a descriptive header about the content of the block. In addition to analog, data physical data include event and image data. The data can be considered as points in time in reference to a master timing generator, and thus data from any block can be easily and rapidly associated with any other block. As the complexity of the data increases, appropriate block header information is added to describe the complexity.

ARCHIVE MEDIA

There are a variety of archival media to choose from in storing data. Large hard drives are becoming so inexpensive now that we sometimes store data on hard drives for pseudopermanent archival purposes. Tape technology has also been improving. In the past, 8-mm tape was the tape of choice. However, after a couple years, significant errors may appear since older 8-mm tape technology used CRC error checking which does not effectively identify some errors. For very large data sets, digital linear tape (DLT) tape technology is the least expensive and most reliable per MB and can sustain 5 MB/s data rates. Redundant arrays of inexpensive disk (RAID) technology allows multiple hard drives or tape drives to be used in parallel, allowing data transfer at the full rate of the interface bus. For our experiments having less than 5 MB/s throughput, we usually store data directly onto tape media. For higher data rates, we use RAID tape arrays, or fast hard drives.

Once written to the archive device, the data no longer need to be accessed from the storage device. The incoming data stream is actually split into two separate streams. One high-performance stream goes directly to the archive media for reliable storage, and the other stream is directed to the various computer processes that display and analyze the data on-line (see Fig. 5).

REAL-TIME CONTROL

During a typical experiment, the investigator often needs to control parameters during data acquisition. A stimulus signal may be generated at specific times during the experiment and feedback may be given in response to certain conditions. For optimal video digitizing, the acquisition system needs to provide the drive signals to the CCD camera in addition to digitizing the

pixel data. We accomplish such control in two ways: (1) during the acquisition of digitized data, a parallel, control bus can output control signals in parallel, and (2) built-in DSPs can output control signals with a coarse time granularity for stimuli and feedback signals.

REAL-TIME DISPLAY AND PROCESSING

Often, data can be collected during the experiment and analyzed later. However, in many circumstances, it is useful or necessary to view some analyzed data as they are being collected. The level of real-time display and processing can be varied with the capabilities of the host computer system. Software that performs such tasks at a variable "gear ratio" work best for such procedures. This approach defines a minimum data unit, such as a data buffer, video frame, or electrophysiological sample, and assigns skip values for each data unit in the stream. The highest priority is to archive the incoming data buffers. Software must accept new data buffers as soon as they are available, and archive them. Computer processor time available between full buffers can be used for data transmission to other processors, data display, and real-time processing. Much like cogs on a gear, data can be displayed at regular intervals during the period between full buffers. If the processor has a lot of time, much of the data can be displayed. If there is not much time, more of the data are skipped.

It is important to display data in a regular interpolated fashion so that the data display does not look "jerky" or distorted in time; to achieve this end, it is important to skip regular integral periods of time. For example, if the current display setting is set to show every frame of a video buffer, and a full buffer arrives before the full sequence is displayed, then the skip value is incremented, and the program displays every other frame for the next buffer. To make the images more evenly displayed across time, the average time between full buffers can be divided by the number of images to display to calculate a time delay factor between images. For truly multithreaded operating systems, it is important that the process goes to sleep during this delay time. Other critical functions can be performed during the delay, such as archiving, processing, and data transmission to other processes.

INTEGRATION OF DATA FROM DIFFERENT MODALITIES

One of the most difficult technical challenges involves integration of data from different sources that need

to be accurately synchronized in time. This includes multiple sources of the same kind of data (i.e., electrophysiological data) or different kinds of data, such as video images and electrophysiology, which need to be time synchronized. Most systems have a difficult time with this procedure, especially for high-rate data streams, because data arrive from different sources with different acquisition clocks. We control acquisition of all data types with the same hardware, thus easing timing and synchronization. Data arrive at the host in the form of packets with header information about the type of data and when it was collected within the

stream. The IFFPHYS file format greatly simplifies interpretation and archiving of such data.

HOST COMPUTER AND USER INTERFACE

We find that the Linux operating system serves well as an operating system for advanced data acquisition. Not only is it free, robust, and powerful, but it can handle many processes quite efficiently with rapid context switching, so that no data buffers are missed. The control software is written using the "C" programming

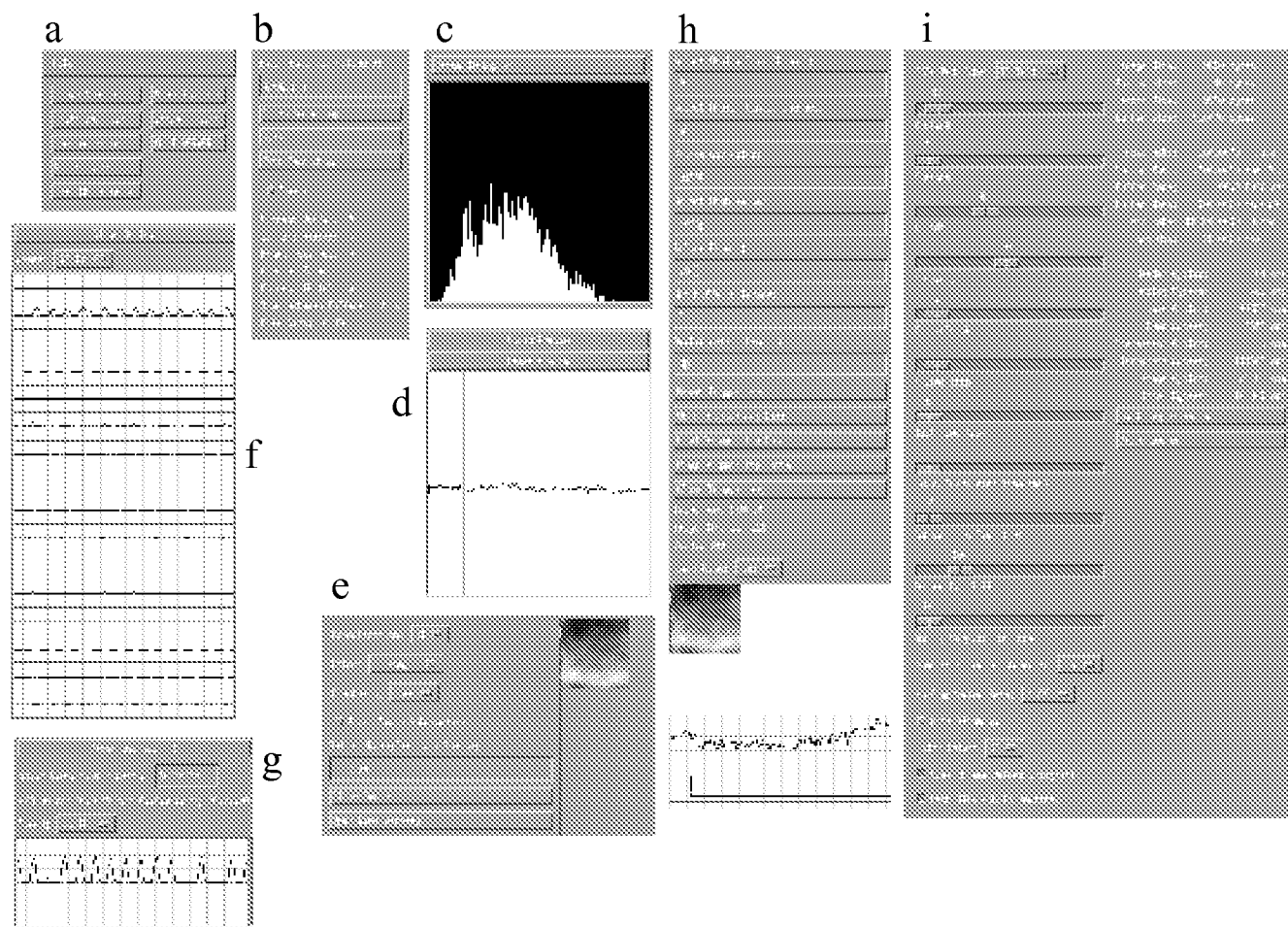


FIG. 4. The user interface for the acquisition and control software uses at least nine basic pop-up windows for the X-Windows interface. Each pop-up window represents an independent process task that can reside within the main host computer, another CPU within the host computer, or other computer on the network. Each pop-up window requests data from the data collection server through internet socket protocols. The Main Server Window (a) is used to initiate other tasks. The Record Window (b) is used to control and display the archive process. The Histogram Window (c) displays pixel intensity histogram profiles for each displayed image. The Preliminary Window (d) plots specific types of analysis; in this case, the average image intensity is plotted. Acquired images can be displayed in many forms in the Image Window (e). Electrophysiological channels are plotted in polygraph form in the Analog Window (f). Each analog channel can be displayed separately as shown in the Channel 1 Window (g). More sophisticated types of on-line analysis can be added, such as the Time Triggered Average Window (h), which configures analysis parameters and displays image sequences and traces. The Loop Control Window (i) is used to program the loop control chip and specify data acquisition parameters.

language and the X-Windows interface is used for creating buttons and widgets to allow the user to interact with all aspects of the program. Separate windows pop up for different aspects of the acquisition; for example, a button on the main window will bring up a new window to display electrophysiological traces in polygraph form, with display rate and amplitude controls for all channels (Fig. 4). The user interface was implemented using the "Lesstif" libraries available for Linux, a free version of the popular "Motif" window interface.

Elements within the user interface are easily modified and appended as they represent separate programs that operate independently as separate process tasks and request data from the main collection server task through internet socket protocols. Thus, to add a feature or have multiple views of the same data, the user can spawn another process to access the data. Since the data are in IFFPHYS format, it is easy to parse the data stream for data relevant to the particular window. Since the user interface uses X-Windows, the entire package is portable across other UNIX platforms including other high-performance hardware platforms (Sun, SGI, HP). This arrangement allows maximum flexibility for performance enhancement at minimal cost. Continued improvements are made with minimal effort and flexibility through network interfaces for display and modular multitasking in an asynchronous parallel computing environment.

The system provides solutions for on-line analysis

and mass storage of continuous data streams through the use of parallel tasking and high-speed network connections. The software collects and stores data; displays images, electrophysiology, and histograms; and performs a variety of simple-to-complex on-line analyses concurrently. Sophisticated post hoc analysis routines use predefined parameters to sort through images and create files for statistical comparison and display.

EVOKED LIGHT SCATTERING CHANGES FROM THE RAT DORSAL BRAINSTEM

We have imaged fast optical changes associated with evoked neural activation in the rat dorsal brainstem. The work employed an endoscopic imager we developed and the acquisition system described above. General surgical and physiological preparation methods have been described previously (15). Twenty-five male rats (250–450 g) were used in separate experiments, typically one per day. Animals were anesthetized with urethane (1.3 g/kg), and the femoral artery and vein were cannulated. A tracheotomy was performed for artificial ventilation, and the right cervical vagus nerve bundle was dissected to identify and isolate the vagus (VN), aortic nerve (AN), and superior laryngeal nerve (SLN) from the surrounding tissue. Animals were placed in a stereotaxic apparatus and suspended from a cervical

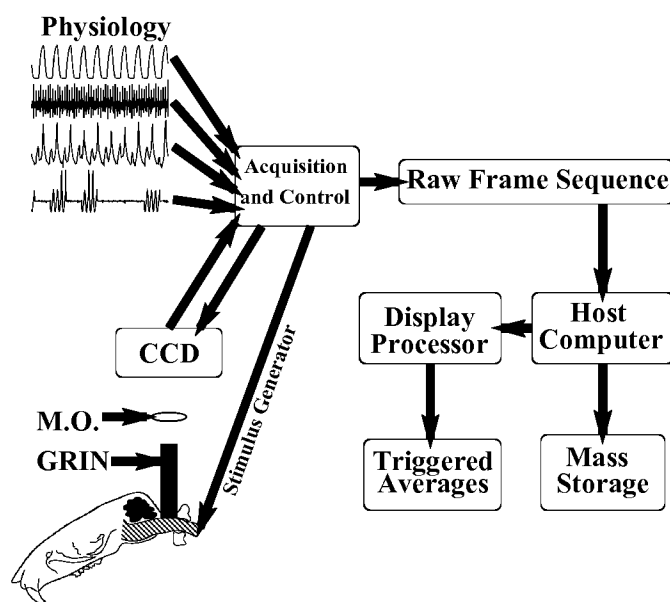


FIG. 5. In these experiments, one remote board is responsible for controlling the CCD camera and stimulus generator, while acquiring electrophysiological data and CCD images. The remote board sends the raw frame sequences and physiology to the host computer through the interface board. The host computer splits the data stream into two duplicate streams. One stream is directed to the mass storage device; the other stream is used for on-line analysis, time-triggered averaging, display.

vertebra. The AN, VN, and SLN were placed intact on bipolar stimulating hook electrodes.

The endoscope probe, described in detail elsewhere (12), was placed on the brainstem surface overlying the NTS (Fig. 5). A series of optical fibers around the perimeter of the probe illuminated the tissue. Backscattered light was collected using a 3-mm-diameter gradient index (GRIN) lens (Gradient Index Corp., Rochester, NY). An image from beyond the end of the probe was focused through a microscope objective lens onto a CCD camera (TC245, Texas Instruments, Dallas, TX). Camera output was sampled by a 12-bit digitizer system at 192×165 pixels, 100 Hz, and simultaneously collected 16 physiological signals (1 kHz each channel), including the electrocardiogram (EKG), tracheal pressure, blood pressure, and end-tidal CO_2 . A fine wire was inserted under the probe for recording evoked responses to nerve shocks. The aggregate data rate was 6.5 MB/s.

To increase sensitivity of very small changes in light intensity, we increased the effective dynamic range of the video digitizing system through judicious selection of black level and gain on the video amplifiers. The black level was first set such that a digital value of "64" represented the dimmest pixel in the image, and a digital value of "4032" represented the brightest pixel in the image. This procedure increased our ability to detect intensity changes by a factor of 6.7 (16). One disadvantage of this procedure, however, is that it requires an extra calibration step to recover absolute intensity values.

After a 30-min stabilization period, the tissue was illuminated with 780-nm monochromatic light from a laser diode array through plastic optical fibers arranged around the perimeter of the GRIN lens. The depth of focus was initially adjusted to $400 \mu\text{m}$ below the surface. Backscattered light images from the NTS were digitized during shocks to different nerves of the vagal bundle: AN, VN, or SLN stimuli were controlled by the acquisition system. Each channel was set to an amplitude twice that required to evoke a reflexive change in heart rate and arterial pressure in response to a 1-s, 100-Hz pulse train. The stimuli were given in random phase relative to the cardiac and ventilatory cycles (typically $40 \mu\text{A}$, 0.1-ms single pulse, 1- to 2-s random interval). Camera frames were digitized continuously (100 fps) along with physiological signals (1 kHz per channel) and multiplexed into the IFFPHYS file format.

The acquisition computer and software displayed images and physiological signals in real time as well as image sequences averaged relative to the stimulator pretrigger pulse. The computer also stored images and physiological records on a mass storage device for subsequent analysis. Image sequences were generated by subtracting the average of nine prestimulus images

from each of 90 poststimulus images. An average response was obtained by averaging 400 to 1000 sequences collected under identical conditions. Images in the average sequence were pseudocolored for display such that cool colors (blue to purple) represented increased light reflectance from the tissue (typically correlated with decreased neural activity), warm colors (yellow to red) represented decreased light reflectance from the tissue (associated with increased neural activity), and green represented no significant change from the first image. For comparison of neural electrical patterns with hemodynamic effects, image sequences were also generated using the cardiac R-wave as the trigger and correlated on a pixel-by-pixel basis to the blood pressure temporal signal.

RESULTS

Shocks to each nerve elicited an electrical presynaptic population spike 30 ms after the stimulus and a postsynaptic population evoked potential that peaked 80 ms after the stimulus. Electrical stimulation produced consistent spatiotemporal patterns of optical changes, which were recorded with the imaging probe. A typical optical response trace to nerve stimulation integrated over the entire image is illustrated in Fig. 6. The time course plot shows rapid light scattering changes that follow the electrical evoked potential within the imaged tissue. Slower temporal components match hemodynamic events occurring with stimulation under the probe. Images from the first 100 ms are illustrated in Fig. 7. A prominent decrease in reflected light (yellow to red region) appears at the bottom of the image within

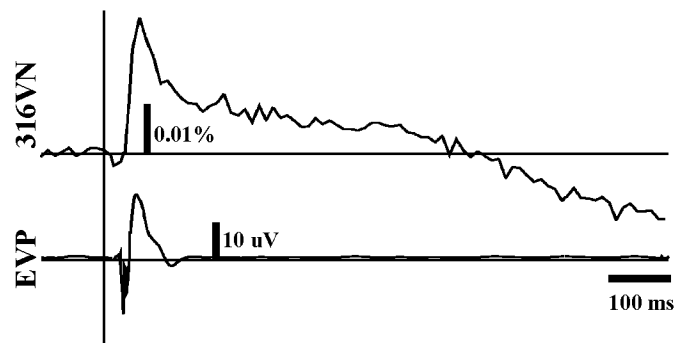


FIG. 6. Time-triggered image sequences were collected over a 1-s period after stimulating the vagus nerve. All pixels of the image were averaged into a single trace to show the time course of light scattering changes. The electrical evoked potential measured from a macrowire placed under the image probe is plotted below the light scattering trace.

three frames (30 ms) after the stimulus and fades away after 140 ms.

Similar patterns were observed for all animals studied, with differences in the spatial organization of the response when stimulating different nerves. Averaged image sequences from the same data sets, triggered by the cardiac R-wave instead of the stimulus pretrigger, were correlated to the blood pressure signal and showed different spatial patterns than stimulus sequences. However, some longer-latency optical responses showed clear similarities with the characteristic hemodynamic patterns.

DISCUSSION

Our needs for high performance and integration of a number of data modalities required that we develop custom data acquisition hardware to control the CCD camera circuitry and digitize video frames, along with many electrophysiological data channels. The host computer consists of one or more Intel Pentium class processors. A fast PCI SCSI card communicates with large and fast hard drives and tape drives. A PCI accelerated display card with at least 8 MB RAM can display images with high speed and quality. A fast ethernet card (100

Mbs or higher) can transfer data rapidly between computers in a multihost system. The PCI interface is used to connect the acquisition board to the host computer.

The high-performance, custom digitizing electronics have programmable flexibility in pixel scanning parameters and allow simultaneous electrophysiological acquisition for 1 to 32,000 channels. The digitizing circuitry stores the data in a double-buffered memory array using an internationally recognized standard file format. Double buffering allows the host computer to process previously collected data simultaneously with acquisition.

These features allow sophisticated optical mapping of light scattering changes associated with neural activation from a large number of neurons at frame rates rapid enough to follow dynamics of the electrical response and to evaluate correlations with other physiological data. In our experience, on-line processing and visualization of data provide feedback that is essential for the optimal conduct of physiological experiments.

Hardware adaptability and speed are gained through use of large field programmable logic arrays in the acquisition circuitry. FPGAs can be reprogrammed on-line for changing experimental conditions or devices, although our present system does not require such

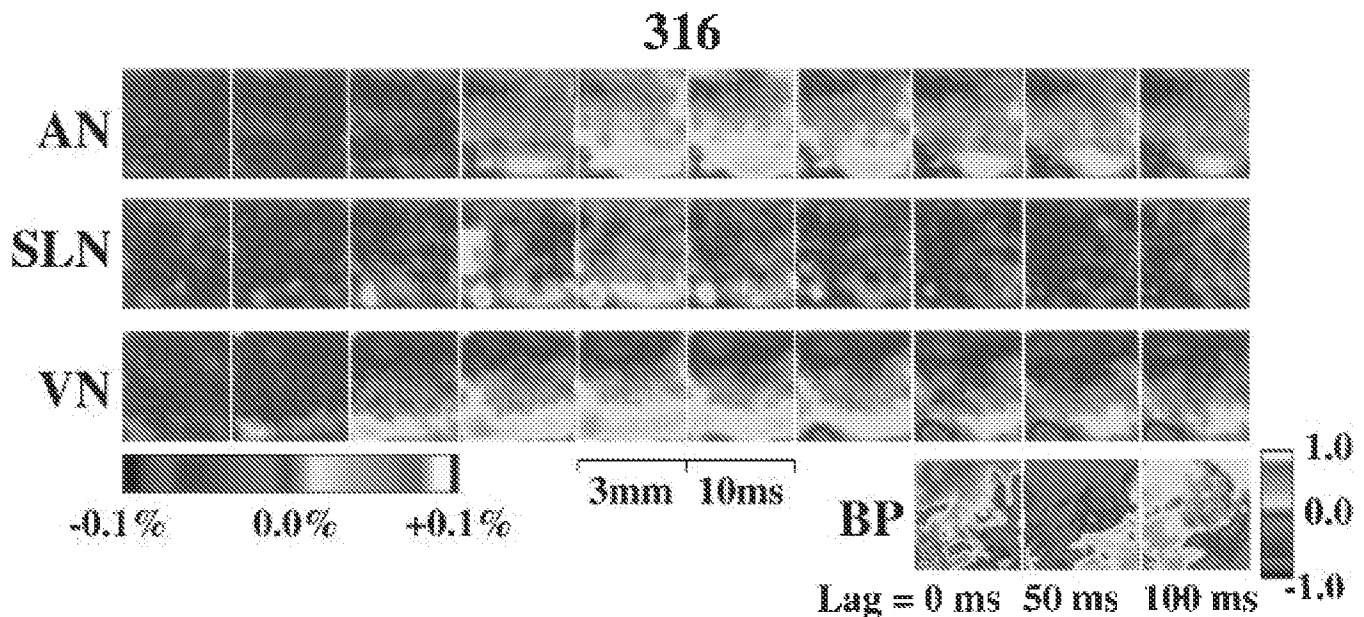


FIG. 7. Three image sequences show the spatial and temporal distribution of scattered light changes in response to stimulating one of three branches of the vagus, superior laryngeal, and aortic nerves. The stimulus begins just before the first image and a 100-ms period is displayed. Each frame is separated by 10 ms; and images are pseudocolored such that warm colors (yellow to red) represent decreased reflectance (increased activation), and cool colors (blue to purple) represent increased reflectance (decreased activity). Green represents no significant change from prestimulus conditions. Each pixel in the acquired sequence is also correlated to the blood pressure signal (BP) and displayed as correlation coefficient intensities for three lag times. The blood pressure correlation images reveal vascular structure under the image probe.

capabilities. We included a dedicated digital signal processor (DSP) to assist in preprocessing the data stream and high-speed communication. High-performance acquisition was achieved through circuitry designed around the PCI bus, which is rapidly becoming the standard for small but powerful personal computers at low cost.

We used the system for recording changes in light scattering occurring in synchrony with evoked potentials in the NTS and observed consistent spatiotemporal patterns in image sequences associated with dorsal medulla activation after nerve shock. Stimulating different branches of the vagal bundle also produced different spatial response patterns.

Previous imaging studies of scattered light changes have failed to observe the fast optical changes reported here. A number of technical improvements have allowed such measurements. Dark-field illumination methods increased our ability to detect small changes in scattered light (17, 18). Bright-field illumination (2) tends to record light reflected from the surface; only a small proportion of the imaged light is perturbed by physical processes associated with activation occurring below the tissue surface. Additionally, blood vessels dominate the tissue surface. More of the bright-field signal is derived from hemodynamic responses to neural activity. Dark-field measurements record deeper changes, since light must first penetrate the tissue before returning to the camera (12, 19).

Increased frame acquisition rates allowed recording of optical changes more rapidly than previously reported, providing finer temporal resolution in the data for recording transient responses. A high-sensitivity CCD camera and amplifiers allowed 12-bit digitizing over the dynamic range of the signal, providing greater sensitivity to small signal changes. Placement of the image collecting gradient index lens in contact with the tissue of interest stabilized the tissue interface, suppressing potential movement artifacts. Focal depth adjustments allowed optimization of signals originating from deep sources.

Since intense illumination levels may affect the tissue through heating or photodamage, final limitations of the CCD camera frame rate depend on the amount of available illumination light provided around the perimeter of the gradient index lens or image conduit. However, coupling of scattered tissue light to the CCD sensors through our probe provides highly efficient detection of backscattered light; nearly all of the returning light is captured. Because very low illumination levels are required, we typically use standard light-emitting diodes as a light source which provide plenty of light for 1-kHz frame acquisition.

We plan a number of improvements to the imaging

probe to increase the spatial and temporal resolution of imaging studies. The probe can be configured to create confocal, spectrally resolved images of the tissue, for better spatial resolution, biochemical and physiological specificity, and 3D reconstruction of tissue activation volume. By using voltage- and calcium-sensitive dyes, it may be possible to identify individual neurons involved in the response, perhaps with even more direct correlations to electrical activation. High-speed and high-sensitivity optical recordings have potential for studying dynamic temporal and spatial activity patterns during cognitive (20) and autonomic (21) functions which may underlie high-level processing within local neural networks. Using near-infrared light, it may be possible to perform low-resolution imaging or tomography of cortical activation noninvasively (6), perhaps in human subjects (22).

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